the mid-19th century (*15*), but the complex interactions between the organisms are still not completely understood. This report emphasizes that point. Current outbreaks of southern pine beetle infestations in eastern Texas and western Louisiana and the inability to predict and control them provide a practical reason for continued study of these fascinating relationships.

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Propolypeptide of von Willebrand Factor Circulates in Blood and Is Identical to von Willebrand Antigen II

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The generally mild bleeding disorder of von Willebrand disease is associated with abnormalities of two distinct plasma proteins, the large multimeric von Willebrand factor (vWF), which mediates platelet adhesion, and von Willebrand antigen II (vW AgII), which is of unknown function. The two proteins were found to have a common biosynthetic origin in endothelial cells and megakaryocytes, which explains their simultaneous absence in the severe form of this hereditary disease. Shared amino acid sequences from a 100-kilodalton plasma glycoprotein and from vW AgII are identical to amino acid sequences predicted from a complementary DNA clone encoding the 5′ end of vWF. In addition, these proteins have identical molecular weights and immunologic cross reactivities. Monoclonal antibodies prepared against both proteins recognize epitopes on the pro-vWF subunit and on a 100-kilodalton protein that are not present on the mature vWF subunit in endothelial cell lysates. In contrast, polyclonal antibodies against vWF recognize both pro-vWF and vWF subunits. Thus, the 100-kilodalton plasma glycoprotein and vW AgII are identical proteins and represent an extremely large propolypeptide that is first cleaved from pro-vWF during intracellular processing and then released into plasma.

VON WILLEBRAND DISEASE (vWD) is characterized by a deficiency or structural defect in von Willebrand factor (vWF), a large glycoprotein that is involved in the binding of platelets to subendothelium after vascular injury and that is the carrier protein for procoagulant factor VIII (antihemophilic factor) (*1, 2*). Von Willebrand factor is present in plasma as a series of disulfide-bonded polymers of 220-kilodalton (kD) subunits (*3*). It is synthesized by megakaryocytes (*4, 5*) and by endothelial cells (*6*), in which it is stored in the endothelial cell-specific organelles, the Weibel-Palade bodies (*7*). In cultured endothelial cells, vWF is synthesized as a fully glycosylated large precursor (*8*) that is processed to the mature subunit (*9, 10*) and assembled into multimers before the protein is secreted (*9, 11*). A full-length complementary DNA (cDNA) for human vWF has been isolated (*12*), and analysis of the 8.15-kilobase (kb) sequence implies that the primary vWF translation product is 300 kD.

Von Willebrand antigen II (vW AgII) is a second protein that is deficient in the plasma and platelets of patients with severe vWD (*13*). It does not share antigenic determinants with vWF, but its deficiency in vWD could be explained by the hypothesis that both proteins are derived from a common precursor (*13, 14*). The antigen is also synthesized by endothelial cells (*14*), is found in the Weibel-Palade bodies (*14*), and is increased in plasma concomitant with vWF, after 1-desamino-8-D-arginine-vasopressin (DDAVP) stimulation (*15*). A complex between vW AgII and vWF is present in endothelial cells, although it is not completely understood (*14*).

A plasma glycoprotein of 100 kD, similar in size to vW AgII, was independently isolated from therapeutic factor VIII concentrates (*16*). Although it was initially believed to be human factor VIII (antihemophilic factor), comparison of partial sequences of this protein (*17*) with that of cloned factor VIII cDNA sequence (*18*) indicates that it is not factor VIII.

We now report that this 100-kD plasma glycoprotein and vW AgII are identical and represent circulating vWF propolypeptide, which is cleaved from pro-vWF subunits during intracellular processing and multimer assembly.

The full-length vWF cDNA is 8.3 kb and has a continuous open reading frame encoding 2813 amino acids (*12*). Amino terminal amino acid sequences of the 100-kD plasma glycoprotein (*17, 19*) and vW AgII (*20*) match amino acid sequences derived from vWF cDNA in clone pVWH33 (*12*), which represents the 5′ portion of vWF cDNA. The region of identity between the two proteins and the sequence predicted by the cDNA begins at nucleotide 67 (Fig. 1). Thrombin cleaves the 100-kD glycoprotein into two fragments of 75 and 26 kD (*16,*

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Intact glycoprotein (100 kD)												
NH_2-terminal sequence	Ala	Glu	Gly	Thr	Arg	Gly	Arg	Ser	Ser	Thr	Ala	Arg
Residue position	1	2	3	4	5	6	7	8	9	10	11	12
Intact vW AgII												
NH_2-terminal sequence	Ala	Glu	Gly	Thr	–	Gly	–	Ser	Ser	Thr	Ala	–
Residue position	1	2	3	4	5	6	7	8	9	10	11	12
VWF cDNA Clone												
5′nucleotide sequence (nucleotides 67 to 102)	GCA	GAA	GGA	ACT	CGC	GGC	AGG	TCA	TCC	ACG	GCC	CGA
Predicted amino acid sequence	Ala	Glu	Gly	Thr	Arg	Gly	Arg	Ser	Ser	Thr	Ala	Arg

Thrombin-Cleavage Fragment (26 kD) of 100-kD glycoprotein																
NH_2-terminal sequence	(Asp) Met	Thr	Arg	Phe	(Ala) Ser	(Pro) Glu	(Ala) Glu	(Val) Ala	(Arg) Cys	Ala	Val	Leu	Thr	(Phe) Ser	Pro
Residue position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
VWF cDNA Clone																
5′ nucleotide sequence (nucleotides 1726 to 1770)	ATG	ACC	AGG	TTC	TCC	GAG	GAG	GCG	TGC	GCG	GTC	CTG	ACG	TCC	CCC	
Predicted amino acid sequence	Met	Thr	Arg	Phe	Ser	Glu	Glu	Ala	Cys	Ala	Val	Leu	Thr	Ser	Pro	

Fig. 1. Comparison of amino acid sequences from a 100-kD plasma glycoprotein and vW AgII with sequences derived from a 5′ cDNA of vWF. Protein sequences were determined by automated Edman degradation. Sequence analysis of the thrombin-cleavage fragment (26 kD) identifies two residues at some positions. The residues shown in parentheses correspond to amino acids that are not predicted from this or other regions of the cDNA sequence, and their origin is not certain.

17). The 75-kD fragment has the same amino terminal sequence as the intact molecule (*17, 21*). The amino terminal amino acid sequence of the 26-kD fragment (*21*) matches the predicted vWF cDNA sequence beginning at nucleotide 1726. Furthermore, the codon preceding this sequence predicts an arginine residue, which is compatible with a thrombin cleavage site (*22*).

The 100-kD plasma glycoprotein and vW AgII were also compared immunologically. Each was immunoprecipitated by monoclonal antibodies developed against purified vW AgII and had a similar molecular weight on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2). Multiple monoclonal antibodies specific for mature vWF did not precipitate this protein, which indicates that this is not a nonspecific reaction. Through the use of polyclonal ^{125}I-labeled antibody to vW AgII, tandem-crossed immunoelectrophoresis of the 100-kD plasma glycoprotein and vW AgII demonstrated a reaction of complete identity (Fig. 3). Together, these results indicate (i) that the 100-kD plasma glycoprotein and vW AgII are the same protein, (ii) that they represent the vWF propolypeptide, and (iii) that the propolypeptide circulates in plasma as a relatively intact protein.

Monoclonal antibodies to the 100-kD protein were used to confirm independently that it is the vWF propolypeptide; similar results were obtained with monoclonal antibodies to vW AgII. Lysates from endothelial cells metabolically labeled with [^{35}S]methionine were treated with protein A Sepharose, to which either polyclonal anti-vWF antibodies or the monoclonal antibodies to the plasma protein were bound (*8*). The results of an examination of the purified material, both reduced and nonreduced, by SDS-PAGE and autoradiography are shown in Fig. 4. Whereas the polyclonal antibodies recognized both the precursor and the mature vWF subunits, the monoclonal antibodies interacted only with the precursor subunit. In addition, the monoclonal antibodies recognized a protein that migrated into the same positions as the purified plasma protein (shown by Coomassie staining), in the reduced (100 kD) and nonreduced (75 kD) forms. The polyclonal and the monoclonal antibodies recognized the precursor dimer molecules, as shown in the nonreduced samples, but the larger multimers were recognized by only the polyclonal anti-vWF antiserum. This result confirms prior observations (*8, 11*) that the cellular vWF multimers larger than dimer size are composed primarily of the processed 220-kD subunits and therefore are not recognized by antibodies directed against the propolypeptide.

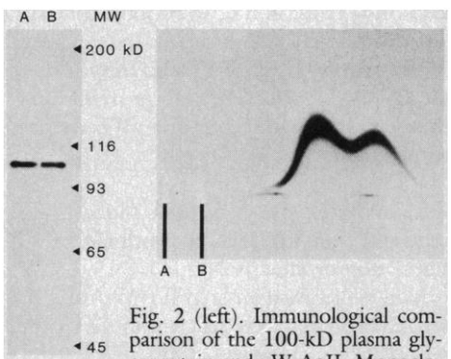


Fig. 2 (left). Immunological comparison of the 100-kD plasma glycoprotein and vW AgII. Monoclonal antibody to vW AgII (MBC 33.5) was used to immunoprecipitate the 100-kD plasma glycoprotein (A) and vW AgII (B). Purified monoclonal antibody was added to each purified protein and immunoprecipitated with protein A Sepharose. The samples were reduced with 10% 2-mercaptoethanol and analyzed on a 5 to 15% gradient polyacrylamide gel stained with silver nitrate. The marks on the right show the migration of standard marker proteins. Fig. 3 (right). Sample of the 100-kD plasma glycoprotein (A) and vW AgII (B) analyzed by tandem-crossed immunoelectrophoresis to compare their antigenic epitopes. The antibody in the second dimension is ^{125}I-labeled polyclonal antibody to platelet vW AgII, and the precipitin arcs were visualized by autoradiography. The proteins gave a reaction of complete immunological identity.

Immunofluorescence staining of permeabilized cultured human umbilical vein endothelial cells with monoclonal antibodies prepared against the 100-kD plasma protein reveals a similar pattern of intracellular staining (Fig. 5), as has been described for antibodies to vWF (*7*) and to vW AgII (*14*). Since most of the vWF in Weibel-Palade bodies contains multimers that consist primarily of mature subunit (*8*), the staining shown in Fig. 4 may represent a reaction with the free vWF propolypeptide.

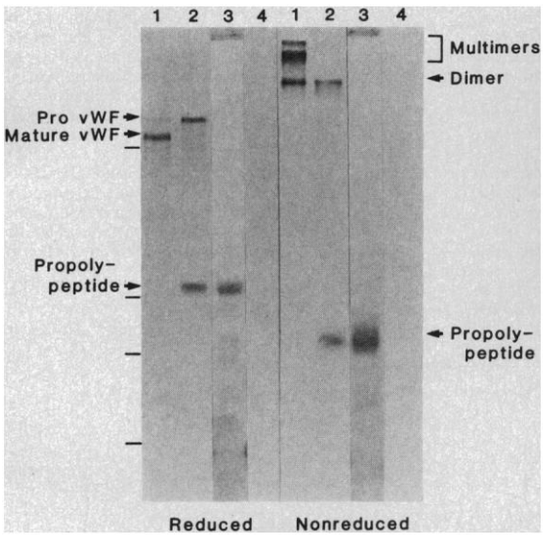
We have shown two previously studied plasma proteins to be identical to each other and to represent the cleaved propolypeptide of the pro-vWF subunit. These conclusions are based on two principal observations. (i) Amino acid sequences of 12 and 15 residues in two different regions of the 100-kD plasma glycoprotein, as well as 9 residues of vW AgII, are identical to sequences derived from a 5′ cDNA clone of vWF (Figs. 1 and 6). (ii) Monoclonal antibodies against this plasma protein recognize only pro-vWF subunits in lysed human endothelial cells and a protein that by its electrophoretic mobility seems to be identical to the original plasma-derived glycoprotein (Fig. 4).

The cDNA clone predicts the size of the precursor vWF monomer to be at least 300 kD (*12*) and the propolypeptide to be about 81 kD, although these do not include the contributions made by carbohydrate side chains (*23*). This result suggests that the precursor subunit of vWF is larger than that estimated by PAGE (275 kD). The latter would predict a propolypeptide of only about 55 kD (275 minus 220) rather than the calculated size of >81 kD (Fig. 6) or the observed size of 100 kD (Figs. 2 to 4). It is

unlikely that additional amino terminal processing takes place to generate a 275-kD form, since the size of the propolypeptide found in plasma (100 kD) is the same as that of the smallest protein identified in the endothelial cell lysate (Fig. 4). The similarity in size of the circulating vWF propolypeptide and that predicted from the prosequence cDNA indicates that a single processing step within the endothelial cell produces the free propolypeptide and the mature vWF subunit (Fig. 6).

In addition to shared amino terminal amino acid sequences, the following observations lead us to the conclusion that the 100-kD plasma glycoprotein is identical to the previously described vW AgII. (i) The two proteins are of the same size as determined by gel electrophoresis (Fig. 2). Both proteins migrated faster without reduction, which indicates the presence of intrachain disulfide bonds, and thrombin cleavage of these proteins produced fragments of identical size. (ii) Both proteins share epitopes recognized by monoclonal and polyclonal antibodies prepared against vW AgII (Fig. 3). (iii) Both are synthesized by endothelial cells and are present in Weibel-Palade bodies (Fig. 5) (*7, 14*). Together with vWF, they are the only proteins of endothelial cells that have been identified within this organelle (*7, 24*). That vW AgII is the vWF propolypeptide is further supported by the observation that a vWF–vW AgII complex and free vW AgII are present in endothelial cell lysates (*14*), most likely corresponding to pro-vWF subunits and to free propolypeptide, respectively (Fig. 4). Since the same processing steps occur in the biosynthesis of vWF by both megakaryocytes and endothelial cells (*5*), the same propolypeptide should be generated by megakaryocytes, an expectation that is consistent with the release of vW AgII from platelet α granules (*25*).

Our principal conclusion that the vWF propolypeptide produced by cleavage from the pro-vWF subunit is not degraded in the endothelial cell and that it is released into the blood along with vWF still leaves several questions of physiological importance unanswered. Thus, although the plasma concentration of the propolypeptide parallels that of vWF in patients with type I vWD, the vW AgII concentration is distinctly higher than that of vWF in patients with type II vWD (*26*). The small vWF multimers characteristic of type II vWD may be more susceptible to intracellular or extracellular degradation (*27*). It has been proposed that the prosequence of pro-vWF is involved in vWF biosynthesis, in particular in the formation of interdimer disulfide bonds (*8*). Whether the circulating vWF propolypeptide has a distinct function of its own remains to be determined. About 1% of vWF subunits that are secreted by endothelial cells in culture contain the prosequence, which is present in multimers of all sizes (*8*), and coagulant factor VIII accounts for about 1% of the total protein of the circulating vWF–factor VIII complex (*1*). These observations suggest that the propolypeptide, perhaps as part of circulating pro-vWF subunit, may transport noncovalently bound factor VIII in plasma.



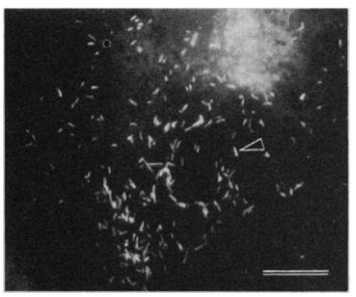


Fig. 4. (left) Endothelial cell proteins recognized by a monoclonal antibody to the 100-kD plasma glycoprotein. Primary cultures of human umbilical vein endothelial cells were grown for 3 days in the presence of [^{35}S]-methionine. The lysed cells were then incubated with protein A Sepharose through the use of bound polyclonal antibodies to plasma vWF (lane 1), monoclonal antibody to the 100-kD plasma protein (lane 2), or protein A Sepharose alone (lane 4). Lane 3 shows the Coomassie blue–stained purified 100-kD plasma protein, loaded onto the same 5 to 10% polyacrylamide gel for which the autoradiograph is presented. All samples were analyzed in the reduced and nonreduced forms. Indications at the left show the migration of markers: myosin, 200 kD; phosphorylase b, 92.5 kD; bovine serum albumin, 69 kD; and ovalbumin, 46 kD. Fig. 5 (right). Epitopes present on the 100-kD plasma glycoprotein are present in the Weibel-Palade bodies of cultured human endothelial cells. Primary cultures of human umbilical vein endothelial cells grown on glass coverslips were fixed, permeabilized, and stained. A monoclonal antibody to the 100-kD plasma glycoprotein was used as the first antibody, and rhodamine-conjugated goat antibody to mouse immunoglobulin G as the second. The arrowhead points to a Weibel-Palade body. Bar, 10 μm.

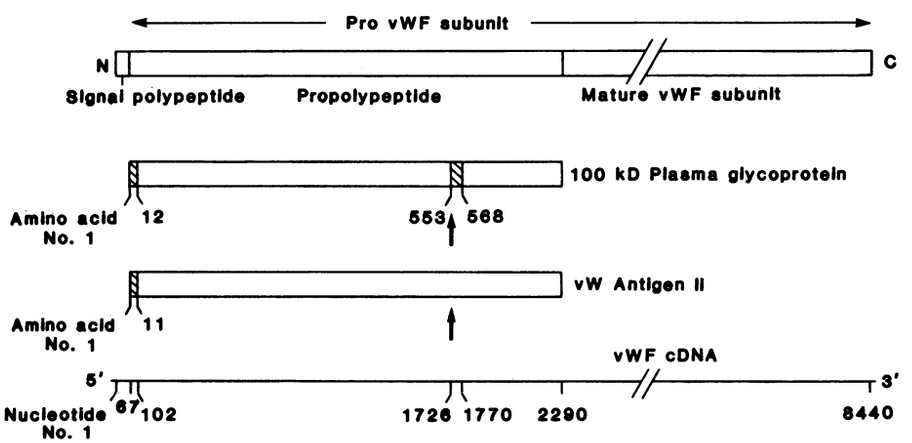


Fig. 6. The relation of the 100-kD polypeptide, vW AgII, and pro-vWF subunit as deduced from-1 sequence studies of the proteins and of the vWF cDNA. Nucleotides 67 to 102 and 1726 to 1770 code for the amino terminal residues determined for the intact (residues 1 to 12) and the 26-kD thrombin-generated fragment (residues 553 to 568) of the 100-kD glycoprotein. Nine amino terminal residues determined for vW AgII are also identical to those of the 100-kD protein and those predicted from the cDNA sequence. The pro-vWF subunit consists of the propolypeptide and the mature vWF subunit and is preceded by a 22–amino acid signal polypeptide (nucleotides 1 to 66). Sequences for the mature vWF subunit begin at nucleotide 2290, as shown by cDNA sequences representing the amino terminal amino acids (*28*). The arrows indicate the thrombin cleavage site in the 100-kD plasma glycoprotein and vW AgII.

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19. This plasma glycoprotein was purified from factor VIII therapeutic concentrates by the methods of Fay *et al.* (*16, 23*). The presence of carbohydrate side chains was inferred from periodic acid–Schiff's (PAS) staining after SDS-PAGE. The amount of protein-bound carbohydrate was estimated by a reduction of the PAS staining intensity and increased electrophoretic mobility after treatment with a mixture of exo- and endoglycosidases (*23*).
20. Plasma vW AgII was purified with monoclonal antibodies to platelet vW AgII. The eluted (diethylamine, *p*H 11) vW AgII was then bound to a Mono-S column (Pharmacia) and eluted with a sodium acetate gradient. After Edman degradation (*29*), the amino acid sequence of vW AgII (0.5 μg, 50 pmol) was determined with a gas phase protein sequencer (Applied Biosystems 470A) according to the method of Hewick *et al.* (*31*). The resultant phenylthiohydantoin amino acids from each cycle were analyzed by HPLC through the use of phenylthiohydantoin analyzer (Applied Biosystems 120A). Amino acids at the 5, 7, and 12 positions were undetectable at this protein concentration.
21. The 100-kD glycoprotein (2.3 mg, 23 nmol) was incubated with human α thrombin (0.9 mg, 23 nmol) in 0.6 ml of buffer containing 20 m*M* imidazole HCl at *p*H 7.0, 150 m*M* NaCl, 2 m*M* $CaCl_2$, and 0.02% NaN_3. Proteolysis of the 100-kD polypeptide was greater than 95% complete after 16 hours at 23°C as determined by SDS-PAGE, which also revealed that the 75- and 26-kD fragments are linked by disulfide bonds. To remove thrombin from the covalently linked fragments, we chromatographed the reaction mixture with Sephacryl S-300 (1 by 31 cm) equilibrated in 20 m*M* imidazole HCl at *p*H 7.0, 0.3*M* NaCl, 0.1*M* lysine HCl, and 0.02% NaN_3 at a flow rate of 5 ml/hour. Fractions (0.5 ml) were collected and monitored for protein. The peak fractions of fragments derived from the 100-kD protein were pooled (1.4 mg, 3 ml). To this mixture was added 1/10 volume of 2*M* tris HCl (*p*H 8.2) and solid guanidine HCl to a final concentration of 6*M*. Dithiothreitol was then added to 10 m*M*, and the reaction mixture was incubated for 1 hour at 23°C. The reduced sample was alkylated with iodoacetamide (11 m*M* final concentration) for 1 hour at 23°C. The reduced and alkylated polypeptides were chromatographed on Sephacryl S-300 (1.5 by 20 cm) equilibrated in 0.1*M* tris-HCl at *p*H 8.2, 6*M* guanidine HCl, 0.15*M* NaCl, 10 m*M* dithiothreitol, and 0.02% NaN_3 at a flow rate of 5 ml/hour. Because of the volume of the sample it was necessary to perform the step in two successive column runs, with less than 2 ml applied per loading. The peak fractions of the purified 75- and 26-kD fragments were conservatively pooled to minimize any cross-contamination. Approximately 890 μg (12 nmol) of the large fragment and 350 μg (13 nmol) of the small fragment were recovered. The fragments were dialyzed extensively against water and lyophilized.
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AIDS Retrovirus (ARV-2) Clone Replicates in Transfected Human and Animal Fibroblasts

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A molecular clone of the AIDS-associated retrovirus (ARV-2) was transfected into human T lymphocyte and monocyte cell lines as well as mouse, mink, monkey, and human fibroblast lines. A replicating virus with cytopathic and biologic properties of ARV-2 was recovered from all the cell lines. The animal and human fibroblast cells are resistant to direct infection by ARV, and in these experiments virus production in the fibroblast lines, especially mouse, was reduced compared to human lymphocytes. However, human fibroblasts were more permissive to virus expression than mouse cells. These results show that, whereas the primary block to ARV infection in certain cells may occur at the cell surface, intracellular mechanisms can also participate in controlling virus replication. The results have relevance to vaccine development and encourage further work with modified molecular clones to examine regions of the ARV genome necessary for cytopathology and replication.

THREE SEPARATE ISOLATES OF THE retrovirus associated with acquired immune deficiency syndrome (AIDS) have been molecularly cloned and sequenced: the lymphadenopathy-associated virus (LAV), the human T cell lymphotropic virus type III (HTLV-III), and the AIDS-associated retrovirus (ARV) (*1*). In most studies of the AIDS retrovirus, investigators have used human T cell lines because other cells are resistant or less susceptible to infection. We now report that after transfection of a molecular clone of ARV-2 into human lymphocytes and into fibroblasts from several mammalian species, replicating ARV can be recovered. The studies indicate that infection by ARV is regulated by intracellular mechanisms as well as cell surface receptors.

The full-length proviral ARV-2 DNA represented in the molecular clone phage λ9B-7 (*2*) was used for these studies. Digestion of this DNA with the restriction endonuclease Eco RI produced two DNA fragments of ARV-2 that were cloned into the Eco RI site of the plasmid vector pSp65 (*3*) (Fig. 1). This plasmid, p9B-7, was also used.

The U937 human monocyte cell line and the Jurkat and HUT-78_{AG} human T cell lines (*4*) were grown in RPMI 1640 medium supplemented with 10 percent fetal calf

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